MRC
Medical Research Council

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reference
Harold E. Varmus, M.D.
& Stephen Hughes Ph.D.
Department of Microbiology,
University of California,
San Francisco,
USA

Dear Harold & Steve,

I think the approach to take on your experiment is to put in a blanket application which would cover both cloning Bam fragments in X1776 and RI fragments in WES or Charon phage, in our category III lab. If this got through, we could get a rapid decision on minor changes, such as a change to the Brenner strain. The latest rumour is that GMAG will be considering its endorsement in February, which could mean that we will have their decision before your application is due to go in. But we should leave other options open.

We have not been involved in any virus applications to GMAG and so I haven't been following their decisions on these. Robin Weiss and Mike Fried would be able to tell you what has been going on. Obviously tumor viruses come high up the list but I wouldn't like to guess where they will place a fragment of an avian tumor virus. I dare say they would be happier if the src gene were deleted but I think it worth while to try to get the application through on wild type. Your arguments, that related sequences are in any case present in mammalian DNA, is double-edged. It does diminish the case for deleting src, but on the other hand it could be used as an argument for up-grading all mammalian shot-gun experiments. The application would be strengthened by detailing the purification steps that will remove most of the src fragments and on this point, a couple of things occur to me. First, would it be possible to include a r-looping step in the purification? I think this would be better than RPC5, and if the RNA were src could be a powerful way of removing src fragments. Second, do any restriction enzymes cut in src but not between the sites you want to use for insertion? This would be an effective way of knocking out the src fragment. It may be necessary for the application to be able to put limits on the amount of residual src DNA, and of course we would have to check colonies for src.

Some points that GMAG will want covered by the application:

Source of the DNA - i.e. details of the rat cells, and the virus of the kind you give in your letter.

The host-vector system.

Details of the experiment, such as what parts will be done in the glove-box, the scale of transfection and how many colonies we intend to screen. (I could do this part if you like). Who will be doing the experimental work and what are their qualifications - they also require people involved to be in some approved health monitoring scheme and we have a medical officer who gives us annual checks, takes blood samples etc. We could include you in this scheme if you do not have some equivalent in the States.

I will send you a sample GMAG form but they are just about to introduce a new streamlined version and I'll make enquires as to when this will be available.

The scientific information you gave in your letter is almost enough for me to fill out a form (a bit more detail on the restriction maps would help e.g. where the cuts are in the cell DNA) and maybe the simplest approach would be for me to fill one out as far as I could go and then send it to you to finish off.

Sorry about all the bureaucracy,

All the best,

Ed

Edwin Southern